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Research Article

Functional responses correspond to stable isotope-based trophic positions among four invasive Ponto-Caspian mysid species (Crustacea, Mysida)

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Abstract

Determining the consumptive effects of non-native predators, for which several direct and indirect methods have been applied, is a common goal in invasion biology. Functional responses and stable isotope analysis are among the most widely used approaches; however, they are rarely used in combination. In this study, we used these two complementary methods to compare the predatory impacts of four invasive Ponto-Caspian mysids on zooplankton in a habitat (Danube River) where all four species coexist. The order of the species based on the attack rates of the functional response models matched with their mean $\delta^{15}N$ values, indicating a correspondence between their predatory potentials and trophic positions in their invaded habitat. *Hemimysis anomala* had the highest attack rate on zooplankton presumably due to its higher degree of specialization linked to its pelagic lifestyle. Contrary to our expectations, the largest species, *Paramysis lacustris*, had an intermediate predatory efficiency and trophic position, similar to those of *Limnomysis benedeni* but higher than those of the smallest species, *Katamysis warpachowskyi*. Nevertheless, all of the four species exhibited a considerable predatory potential, suggesting that any of them can contribute significantly to their combined predatory impact depending on their densities. The congruence between the results of the two methods shows that the species can realize their different predatory potentials in their invaded environment, indicating favorable conditions (i.e., food availability and spatial heterogeneity) which allowed dietary differentiation. We recommend the combined use of functional responses and stable isotope analysis, which might allow robust conclusions to be drawn on the trophic ecology of the species and also provide further insights into the studied ecosystem.

Key words: *Hemimysis anomala*, *Katamysis warpachowskyi*, *Limnomysis benedeni*, *Paramysis lacustris*, predatory impact

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Introduction

Many of the most notorious cases of biological invasions are related to predatory impacts causing drastic declines or even extinctions of native prey populations (Dick and Platvoet 2000; Wiles et al. 2003; Doherty et al. 2017). The reason behind this lies in the inherently dynamic nature of predator-prey interactions, often

resulting in stronger and more immediate effects than for example in cases of resource competition (Sih et al. 2010). Although prey species can develop anti-predator defenses, these also incur costs on them, implying often considerable non-consumptive negative effects (Sih et al. 2010; Jermacz and Kobak 2017; Kindinger and Albins 2017). Nonetheless, the most dramatic impacts are usually caused by the direct consumption of prey, especially where naïve species lacking effective antipredator mechanisms are concerned (Cox and Lima 2006; Sih et al. 2010). The decline of prey populations can often propagate through the food webs via indirect interactions (e.g., trophic cascading), leading to an often considerably altered new ecosystem state (Ricciardi et al. 2012; Walsh et al. 2016; David et al. 2017).

Determining the consumptive effects of non-native predators is a common goal in invasion biology. Since the direct visual analysis of stomach contents is often problematic (especially in small animals with masticatory organs) and burdened heavily with spatiotemporal variation, several indirect methods have been developed (Nielsen et al. 2018). One of the most popular approaches is the experimental determination of the predators' functional responses; i.e., the per capita predation rates as a function of their density (Holling 1959), which has recently even been proposed as a unifying framework for invasion biology (Dick et al. 2017; Faria et al. 2023). Another widely used method is the determination of stable isotope ratios of prey and consumer species (Post 2002; Jackson et al. 2011; Phillips et al. 2014; McCue et al. 2020). The stable isotope ratio of carbon ($\delta^{13}C$) can be used to estimate the relative contribution of littoral (benthic algae and detritus) and pelagic (phytoplankton) primary production to the carbon assimilated by consumers, while the stable isotope ratio of nitrogen ($\delta^{15}N$) is informative of their trophic positions (i.e., it can be used as a proxy to estimate their mean trophic levels; Post 2002; Jackson et al. 2011; Phillips et al. 2014). Despite their overlapping objectives, these two methods provide insights into somewhat different aspects of the trophic ecology of consumers. Functional responses provide a quantitative estimation of the strength of specific predator-prey interactions. On the contrary, stable isotope ratios are informative of the diet sources and relative trophic positions of consumers realized in their environments over a longer time period.

Although both methods are widely used, they are rarely combined (Rosewarne et al. 2016; Dominguez Almela et al. 2021). As these methods all come with advantages and limitations, their complementary use may result in a more robust estimation on the trophic roles of invasive predatory species. In addition, due to their different interpretations, the comparison of the two results might also provide further insights. In the present study, we combined these two methods to gain a deeper understanding of the predatory impact of invasive Ponto-Caspian mysids.

Owing to its highly diverse endemic euryhaline fauna, the Ponto-Caspian region (i.e., the Black, Azov, Caspian, and Aral seas and the lower sections of the rivers running into them) is one of the most important sources of aquatic invasive species in Europe and in North America (Ricciardi and MacIsaac 2000; Bij de Vaate et al. 2002). The majority of the invaders are peracarid crustaceans (Borza et al. 2017; Copilaș-Ciocianu et al. 2023), including representatives of the order Mysida. Since the middle of the 20th century, four Ponto-Caspian mysid species have expanded their ranges in Central, Western, and Northern Europe (Bij de Vaate et al. 2002; Borza et al. 2017), while one of the species is also present in the British Isles as well as in North America (Audzijonyte et al. 2008). These relatively small (reaching ~0.5–1.5 cm body length), omnivorous crustaceans can consume

small suspended particles by filtering (Borza et al. 2023a, 2023b) as well as deposited materials and biofilm. However, their invasion impact is attributable primarily to their ability of grabbing larger prey items such as zooplankton and benthic invertebrates (Ketelaars et al. 1999; Lesutienė et al. 2007; Fink et al. 2012).

Previous studies suggest that the predatory impacts of the four species might be different. The most widespread species, *Hemimysis anomala* G. O. Sars, 1907 is the most notorious for its partially predatory diet causing substantial changes in the zooplankton community, thereby altering pelagic trophic pathways (Ketelaars et al. 1999; Borcherding et al. 2006; Ives et al. 2013; Evans et al. 2018). *Paramysis lacustris* (Czerniavsky, 1882), the largest of the four species, has also been identified as an omnivorous species with high potential for consuming animal prey, including both zooplankton and benthic macroinvertebrates (Lesutienė et al. 2007; 2008, Rakauskas 2019). *Limnomysis benedeni* Czerniavsky, 1882 is generally considered less predatory than the two species mentioned above (Fink and Harrod 2013; Hanselmann et al. 2013; Rothhaupt et al. 2014); nevertheless, its ability for catching animal prey and even changing community composition has been demonstrated under experimental conditions (Fink et al. 2012; Patonai et al. 2024). The smallest of the four species, *Katamysis warpachowskyi* G. O. Sars, 1893 was found to consume animal prey only marginally (Wittmann 2002). In Lake Constance, its trophic position was similar to that of *L. benedeni* (Rothhaupt et al. 2014).

These dietary profiles are based mainly on single-species studies (with the exception of Rothhaupt et al. 2014 comparing *L. benedeni* and *K. warpachowskyi*). Although the four species coexist in their native range as well as in the Middle Danube, their predatory impact has not been studied comparatively in sympatric populations. To address this knowledge gap in a robust way, we compared the predatory impacts of the four invasive Ponto-Caspian mysids based on two complementary methods: (1) their functional responses to zooplankton prey in a laboratory experiment and (2) stable isotope ratios from a habitat where all four species coexist. Based on the generally positive relationship between the body sizes of predators and their prey (Hansen et al. 1994; Barnes et al. 2010) and the often observed ontogenetic shift towards animal prey in the diet of mysids (e.g., Borcherding et al. 2006; Lesutienė et al. 2007), we expected *P. lacustris*, the largest of the four species, to be the most predatory. Based on previous studies (e.g., Ketelaars et al. 1999; Hanselmann et al. 2013), we also expected *H. anomala* to be more predatory than the similar-sized *L. benedeni*, followed by the smallest species, *K. warpachowskyi*.

Materials and methods

Functional response experiment

The specimens of the four mysid species used for the experimental test of functional responses were collected on 29.10.2019 in a semi-enclosed inlet of the Danube River) within the city of Budapest, Hungary ('Lágymányosi-öböl'; 47°27.9'N, 19°03.6'E) by a hand net. The populations of the species at the time of the sampling consisted mainly of relatively large (i.e., similarly-sized as the adults of the summer generations) but sexually immature specimens of the overwintering generation with a few mature individuals originating from the last summer generation (Borza 2014). The collected specimens (Table 1) were transported to the lab in water taken from the sampling location. Subsequently, the specimens were placed

Table 1. The setup of the functional response experiment. The body length and dry weight values represent the means and ranges (in brackets).

Predator					Prey density (replicates)
Species	No. of specimens	Body length, mm	Dry weight, mg	Sex	
Hemimysis anomala	24	6.0 (5.0–8.0)	1.2 (0.6–1.7)	1 male, 23 juv.	2(3), 4(3), 6(3), 8(3), 10(3), 15(3), 20(3), 30(3)
Katamysis warpachowskyi	10	4.5 (3.5–5.0)	0.4 (0.2–0.6)	4 females, 6 juv.	2(1), 4(1), 6(2), 8(3), 10(3)
Limnomysis benedeni	12	6.25 (5.5–7.0)	1.1 (0.8–1.3)	12 juv.	2(1), 4(1), 8(1), 10(3), 15(3), 20(3)
Paramysis lacustris	24	9.5 (7.0–11.0)	2.65 (1.6–4.4)	3 males, 21 juv.	2(3), 4(3), 6(3), 8(3), 10(3), 15(3), 20(3), 30(3)
None (control)					2(1), 4(1), 6(1), 8(1), 10(1), 15(1), 20(1), 30(1)

individually in 200 ml jars filled with dechlorinated tap water without food and allowed to acclimatize for 24 hours at 23 °C (a temperature that was kept constant during the whole experiment).

At the start of the experiment, commercially available *Daphnia pulex* Leydig, 1860 (body length: 1.5–2 mm) were added to the jars as prey corresponding to the experimental prey density levels (Table 1). Since we unfortunately could not collect the desired number of specimens (N = 24) in the case of *L. benenedeni* and *K. warpachowskyi*, fewer levels of prey densities were applied in these two species chosen based on prior expectations of consumption (Table 1). The feeding experiments were run for 12 hours in the dark, after which the remaining prey items were counted, and the body lengths and dry weights of the mysids were measured.

Stable isotope analysis

The samples for stable isotope analysis were collected in the same location as mentioned above between 29.-30.08.2022. In total, samples for seven different food web components were obtained: four mysid species, Copepoda, Cladocera, and detritus. The four mysid species were collected using a hand net and sorted by species into separate containers in the field. Zooplankton samples were collected using a 70 μm-mesh-sized plankton tow net, rinsed with distilled water, and stored in plastic containers. Pieces of decomposing willow leaves (i.e., detritus) were picked from the sediment material caught up in the hand net during the collection of the mysids. Zooplankton and detritus samples were stored on dry ice until returning to the laboratory where they were stored at –20 °C until further processing. In the laboratory, the mysid specimens were kept in filtered tap water over gauze for 24 hours to allow their gut contents to empty before freezing (Rothhaupt et al. 2014).

For each stable isotope sample of animals, entire individuals belonging to the same taxonomic group were processed as a batch, yielding 4–6 sample replicates per group. Mysid samples contained 4–5 (*P. lacustris*) or 8–11 (the other three species) adult individuals of both sexes distributed evenly (corresponding to the sex ratio of the collected material). Cladocera and Copepoda (copepodites and adults of calanoids and cyclopoids, excluding nauplii) samples contained several specimens sorted using a fine tweezer. All samples were placed in 2 ml cryovials covered with lint-free tissue paper held by rubber bands and freeze-dried for 24 hours.

Lipids are more depleted in ^{13}C compared to carbohydrates and proteins (DeNiro and Epstein 1977; Griffiths 1991). Consequently, as the lipid content can vary considerably among organisms as well as temporarily, it may bias the interpretation of trophic relationships (Arts et al. 1993; Matthews and Mazumder 2003; Murry

et al. 2006). To overcome this, chemical lipid extraction is frequently applied on samples being analyzed for $\delta^{13}C$. However, since the pre-treatment of lipid removal can significantly affect the $\delta^{15}N$ value – the main focus of our study as an indicator of trophic position (Sweeting et al. 2006), we did not perform chemical extraction of lipids. Instead, we applied arithmetic correction following Post et al. (2007). Freeze-dried and homogenized specimens were placed into tin capsules (~0.5 mg dry weight per sample) and analyzed using an elemental analysis isotope ratio mass spectrometer (EA-IRMS; Elemental Analyzer Thermo Scientific™ FLASH 2000 HT™, USA).

Data analysis

Functional response models were fitted using the 'frair' package (Pritchard et al. 2017) in R 4.3.1 (R Core Team 2023). Since the model selection tool 'frair_test' (implementing a forward selection based on the sign and significance of first and second-order terms in logistic regressions; Juliano 2001; Pritchard et al. 2017) did not give a clear indication of the preferred model type, Type II models were chosen based on the visual inspection of the data and on previous studies showing its prevalence in mysids (Mohammadian et al. 1997; Dick et al. 2013; Barrios-O'Neill et al. 2014). The coefficients a (attack rate; i.e., the initial slope of the curve) and h (handling time; $1/h$ corresponding to the asymptote of the curve) were estimated based on the model accounting for prey depletion using maximum likelihood estimation (response = 'rogersII' in the 'frair_fit' function; Rogers 1972; Pritchard et al. 2017). The 95% confidence intervals of the parameters were determined by bootstrapping ('frair_boot') with 999 iterations. Interspecific comparisons of coefficients were made by the difference method (Juliano 2001) implemented in the 'frair_compare' function (Pritchard et al. 2017). To account for the potential differences between developmental stages, the analysis was repeated without the mature specimens. However, since the exclusion resulted in quantitatively similar outcomes with no significant differences compared to the original models (Suppl. material 1), the results based on the full dataset are reported.

Our aquatic invertebrate samples had high C:N ratio (>3.5), thus lipid correction was applied using the equation for aquatic animals ($\delta^{13}C_{corr} = \delta^{13}C_{raw} - 3.32 + 0.99 \times C{:}N$; Post et al. 2007), where $\delta^{13}C_{raw}$ refers to the raw measurement, C:N is the carbon-to-nitrogen ratio per sample, and $\delta^{13}C_{corr}$ is the lipid-corrected value. Detritus was corrected using the equation for plant samples for carbon content over 40% ($\delta^{13}C_{corr} = -5.83 + 0.14 \times$ % Carbon; Post et al. 2007). Subsequently, $\delta^{15}N_{raw}$ values were plotted against the lipid-corrected $\delta^{13}C_{corr}$ values to visualize the trophic position of each food web component. To reveal significant differences in the isotopic signatures (for $\delta^{13}C_{corr}$ and $\delta^{15}N_{raw}$ values, respectively) of the four species, Kruskal-Wallis test and Dunn's multiple comparison post hoc test with Holm adjustment of p-values was performed using the 'dunn.test' package (Dinno 2017). Besides, the isotopic niche for the 4 species of mysids was determined using the Stable Isotope Bayesian Ellipses (SIBER) package (Jackson et al. 2011). Due to the relatively small sample sizes (N = 6 for *P. lacustris*, N = 5 for the other three species), a correction was applied during the calculation of standard ellipse areas (SEAc; Jackson et al. 2011). For each species pair, the area of overlap based on a prediction interval of 95% was calculated using the 'maxLikOverlap' function in the SIBER package (Jackson et al. 2011). Overlap percentage (%) was calculated in terms of the overall area occupied by the two species ($A \cap B / A \cup B * 100$).

Results

Functional response experiment

Three of the four studied species – *H. anomala*, *L. benedeni*, and *P. lacustris* – showed density dependent consumption of *D. pulex* during the experiment (Fig. 1). Since consumption did not reach a clear plateau at the highest prey densities in any of the mysid species, the handling time parameter (*h*) of the fitted Type II functional response curves could not be estimated with sufficient statistical power (Table 2). However, the attack rates (*a*) allowed interspecific comparisons, showing that *H. anomala* had significantly higher consumption in the pre-saturation phase of the curves than either of the other two species, whereas the difference between *L. benedeni* and *P. lacustris* was not significant (Table 3).

In the case of *K. warpachowskyi*, only 1–2 *D. pulex* specimens were consumed in eight out of the ten jars independently of prey density (Fig. 1). Nonetheless, the overall mortality of prey items in the presence of the species (19.4%) exceeded background mortality in the control jars (7.4%) significantly (Fisher's exact test, $p = 0.04$).

Table 2. Estimates of the parameters of the Type II functional response curves (*a*: attack rate, *h*: handling time). The units of the parameters correspond to the experimental setup (*a*: 1/200 ml/12 hours; *h*: 12 hours).

Species	Parameter	Estimate	Lower CI	Upper CI
Hemimysis anomala	*a*	1.459	1.029	2.198
	h	0.02	0	0.052
Limnomysis benedeni	*a*	0.515	0.335	0.703
	h	0	0	0.139
Paramysis lacustris	*a*	0.693	0.517	1.074
	h	0.027	0	0.075

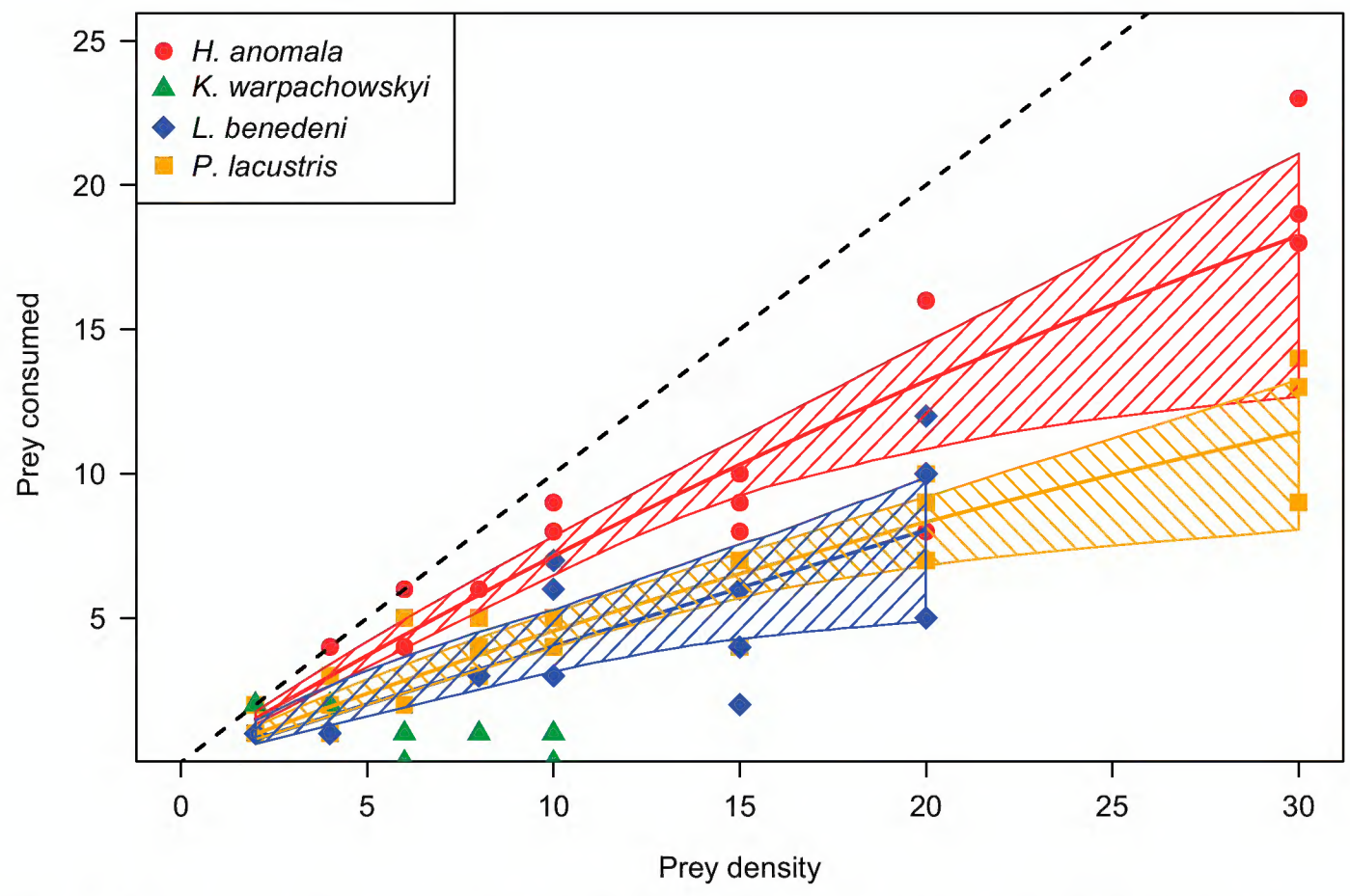

Figure 1. Functional responses of the four invasive Ponto-Caspian mysid species. The solid lines correspond to the fitted Type II curves with the 95% confidence intervals indicated by the dashed areas. The dashed line indicates the consumption of all prey items (slope = 1).

Table 3. Estimates of the interspecific differences between the parameters of the Type II functional response curves (*a*: attack rate, *h*: handling time). The units of the parameters correspond to the experimental setup (*a*: 1/200 ml/12 hours; *h*: 12 hours).

Compared species	Parameter	Estimate	SE	*Z*	*p*
H. anomala – *L. benedeni*	Δa	0.946	0.352	2.687	0.0072**
	Δh	0.020	0.071	0.287	0.7742
H. anomala – *P. lacustris*	Δa	0.766	0.308	2.487	0.0129*
	Δh	-0.007	0.025	0.287	0.7744
P. lacustris – *L. benedeni*	Δa	0.180	0.276	0.653	0.5139
	Δh	0.028	0.074	0.373	0.7090

Stable isotope analysis

Based on $\delta^{13}C$, all investigated animal groups (range: -30.52 to -32.76‰) separated clearly from detritus (-28.76 to -29.83‰; Fig. 2A). Among the four mysids, the two benthic species (*K. warpachowskyi* and *P. lacustris*) showed a somewhat stronger association with detritus than the pelagic *H. anomala* and the semi-pelagic *L. benedeni* when considering mean values (Fig. 2). However, the $\delta^{13}C$ values of *P. lacustris* samples overlapped with the more pelagic species. Accordingly, the Kruskal-Wallis test (χ^2 = 10.035, *df* = 3, p = 0.019) indicated significant differences only between *K. warpachowskyi* and *H. anomala*, and *K. warpachowskyi* and *L. benedeni* (Table 4). The $\delta^{13}C$ ranges of cladocerans and copepods overlapped with those of the benthic mysid species (Fig. 2A).

The $\delta^{15}N$ values were the lowest in the detritus samples (3.92 to 5.89‰), followed by cladocerans (10.13 to 10.42‰), copepods (11.33 to 14.23‰), and mysids (14.24 to 17.36‰; Fig. 2A). Among the four mysid species, *K. warpachowskyi* had the lowest and *H. anomala* the highest mean $\delta^{15}N$ values, while *L. benedeni* and *P. lacustris* occupied an intermediate position with generally higher variation among samples. The post hoc Dunn's test of the Kruskal-Wallis test (χ^2 = 10.736, df = 3, p = 0.013) indicated significant differences only between *K. warpachowskyi* and *H. anomala* (Table 4).

The 95% Bayesian ellipses overlapped between all species pairs except for *K. warpachowskyi* and *H. anomala* (Fig. 2B, Table 4). The highest overlap was observed between the two more pelagic species (*H. anomala* and *L. benedeni*, 32%), followed by the two benthic species (*P. lacustris* and *K. warpachowskyi*, 28%).

Table 4. Results of the post hoc Dunn's test on the stable isotope ratios and isotopic niche overlaps of the four invasive Ponto-Caspian mysid species.

Compared species	$\delta^{13}C$		$\delta^{15}N$		Overlap (95% SEAc ellipses)
	z	p_{adj}	*z*	p_{adj}	
H. anomala – *K. warpachowskyi*	-2.650	0.040*	3.112	0.011*	0%
H. anomala – *L. benedeni*	0.102	0.919	1.020	0.923	32%
K. warpachowskyi – *L. benedeni*	2.752	0.036*	-2.092	0.146	13%
H. anomala – *P. lacustris*	-1.322	0.372	0.786	0.864	19%
K. warpachowskyi – *P. lacustris*	1.446	0.593	-2.464	0.069	28%
L. benedeni – *P. lacustris*	-1.428	0.460	-0.280	0.780	23%

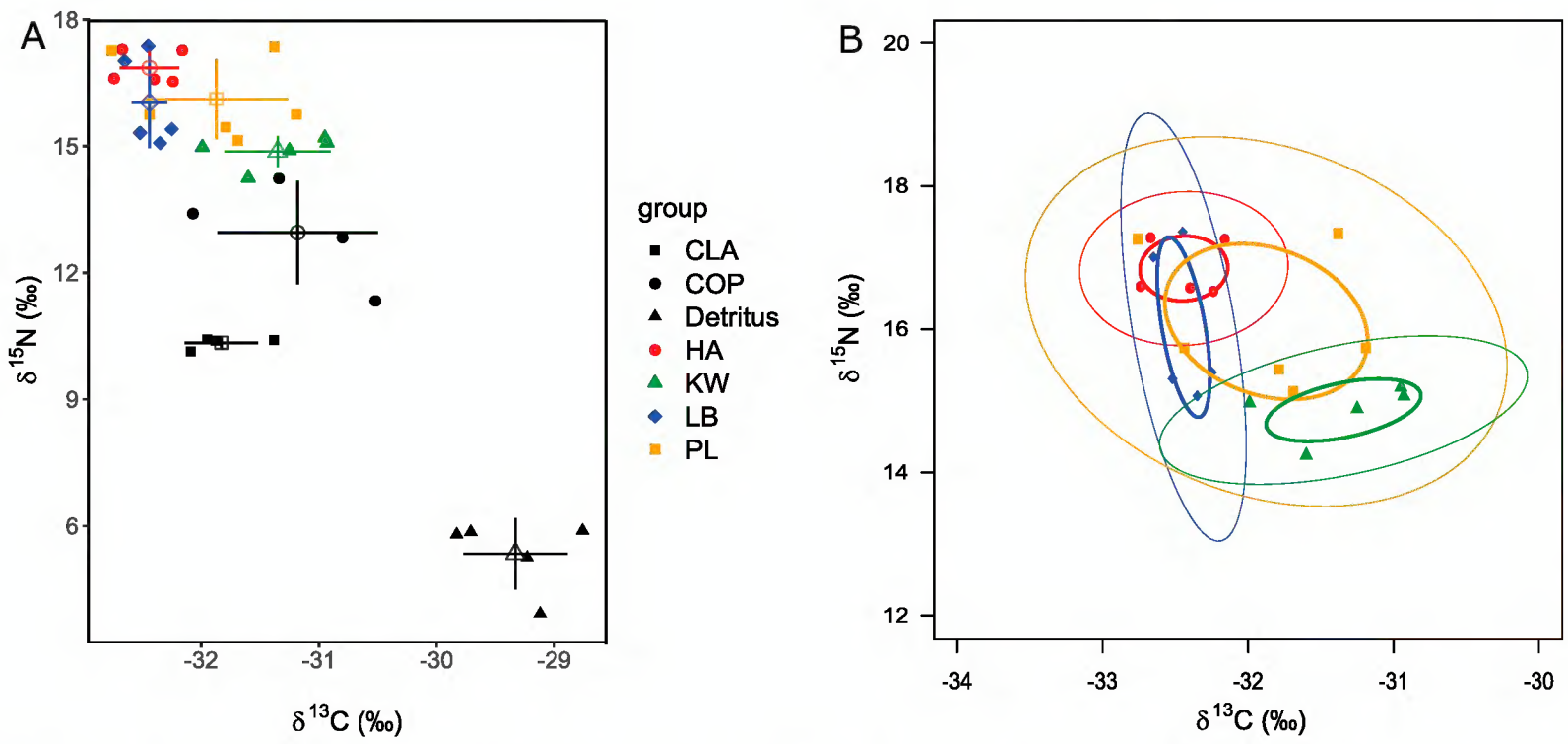

Figure 2. Stable isotope ratios of the studied food web components **A** all groups with means ± SD (empty symbols and error bars) **B** the isotopic niche overlap of the four invasive Ponto-Caspian mysid species illustrated by Stable Isotope Bayesian Ellipses corrected for small sample size (SEAc). The inner ellipses correspond to the 40%, the outer ones to the 95% credible intervals. CLA: Cladocera, COP: Copepoda, HA: *Hemimysis anomala*, KW: *Katamysis warpachowskyi*, LB: *Limnomysis benedeni*, PL: *Paramysis lacustris*.

Discussion

The two methods applied in our study yielded congruent results, providing a consistent picture about the predatory impacts of the four invasive Ponto-Caspian mysid species. Specifically, the order of the species based on the attack rates of the functional response curves matched with their mean $\delta^{15}N$ values, indicating a correspondence between their predatory potentials and trophic positions in their natural habitat. Contrary to our expectations, both approaches indicated *H. anomala* as the most predatory species, whereas the largest species, *P. lacustris*, had an intermediate attack rate and trophic position similar to those of *L. benedeni*. The smallest species, *K. warpachowskyi*, showed the lowest predatory activity in both tests which matched our *a priori* assumptions. The $\delta^{13}C$ values indicated further differences in the diet of the species corresponding to their habitat preferences (benthic or pelagic) which were also reflected in the isotopic overlaps.

The higher attack rate of *H. anomala* indicates that it is more effective at capturing zooplankton than the other species, presumably due to its higher degree of specialization in connection to its pelagic lifestyle. The constant swimming might imply a higher encounter rate which in itself could potentially explain the differences; however, other behavioral, physiological, or morphological adaptations related to prey capture might also be involved (e.g., longer setae on the thoracic endopods; pers. obs.). On the other hand, the active lifestyle of *H. anomala* might also result in a higher metabolic rate, implying that the high ratio of nutritious animal prey in its diet is an energetic necessity in this species. Body size proved to be irrelevant in this context, suggesting that its role in intraspecific diet shifts might be related to prey size limitations that are not relevant at the size of adults. Although we did not test it, it is reasonable to assume that *P. lacustris* is in turn more effective at capturing benthic prey and indeed consumes more of them as indicated by its higher $\delta^{13}C$ values, implying a somewhat stronger association with detritus-based food sources. Nevertheless, the congruence between the results of the two methods suggests that its attack rate on zooplankton reflects its overall trophic position. Unfortunately, our data did not allow the estimation of handling

times which could be used to calculate the maximum feeding rates of the species. However, the higher trophic position and the presumably higher metabolic rate of *H. anomala* suggest that it might potentially have a higher *per capita* predatory impact than *P. lacustris* despite the larger body size of the latter.

Our results on *L. benedeni* confirmed its considerable predatory potential observed previously under artificial conditions (Fink et al. 2012; Patonai et al. 2024), since it had an attack rate similar to that of *P. lacustris*. Moreover, our stable isotope data provided the first indication that animal prey can play a decisive role in its diet also in nature, since its trophic position was considerably higher than that of the obligate filter feeder cladocerans and also somewhat higher than that of the omnivorous copepods. Our results are in contrast with the observations by Fink and Harrod (2013), who found that *L. benedeni* had a lower trophic position than copepods and in one case also lower than cladocerans. Hanselmann et al. (2013) reported a trophic position for *L. benedeni* higher than that of cladocerans; however, copepods were not included in their study. These results altogether indicate high dietary flexibility and related differences in functional roles of *L. benedeni*, similar in range to *H. anomala* and *P. lacustris*.

K. warpachowskyi did not show density dependent consumption in our functional response experiment which might indicate that the size of the offered prey items was close to the upper limit of its potential prey size range. Nonetheless, the significantly higher mortality of *D. pulex* in the presence of the species compared to the control jars suggests that *K. warpachowskyi* was able to actively hunt down at least some of the prey instead of merely consuming the dead ones. The stable isotope data also confirmed that predation can have a considerable contribution to the diet of the species, as its trophic position – although the lowest among the four mysid species – was still somewhat higher than that of copepods. This suggests that *K. warpachowskyi* may still be an effective predator of smaller zooplankton and zoobenthos.

Overall, our results indicate that *H. anomala* is the most effective predator of the four invasive Ponto-Caspian mysids. However, all of the other three species also have a considerable predatory potential, suggesting that their combined effect might be even stronger when occurring in syntopy. The densities of each mysid species might be highly dependent on local environmental conditions (e.g., habitat availability), implying that any of them may become the most impactful in certain areas regardless of their *per capita* effects. We also would like to point out that animal prey is only one component of the diet of these omnivorous species, and other food sources such as phytoplankton, phytobenthos or detritus (Lesutienė et al. 2008; Fink and Harrod 2013; Rothhaupt et al. 2014; Borza et al. 2023b) also might convey important consumptive effects depending on the circumstances.

Beyond the characterization of the species, the combination of the two methods also allows certain conclusions to be drawn on the studied ecological system. The differences in predatory effectiveness among the species revealed by their functional responses can be interpreted as a result of coevolution which can contribute to their niche differentiation. The congruence between the results of the two methods indicates that the species can realize their different predatory potentials in their natural environment. The favorable conditions of the studied water body (e.g., availability of alternative food sources and sufficient habitat diversity) could have allowed the diversification of the diets among the species, indicating a stable coexistence. The differentiation in carbon source between the benthic and pelagic species resulting in lower isotopic overlaps also points in this direction. Alterna-

tively, it can also be presumed that the species are subject to top-down control (by e.g. fish) resulting in relatively low population densities, in which case the food is not a limiting factor for them in the studied system.

Due to the low number of studies applying both methods in parallel, our understanding of the factors determining the connection between functional responses and stable isotope-based trophic positions is still rather limited. It seems likely that the congruent results in our case are not exceptional and similar outcomes can be expected in systems in a near-equilibrium state (i.e., after the establishment and stabilization of invasive populations, as in the present case). However, we expect that discrepancies are possible also among species with coevolved differences in predatory efficiency under certain conditions. For example, predation risks or low food availability might prevent a more effective predator from realizing its potential. Anomalies might be more frequent in cases involving native and invasive species, or multiple invasives with different origins. For instance, one of two species might be competitively superior and therefore more successful at capturing the preferred prey even if their predatory potentials are similar. The study of Rosewarne et al. (2016) covered such a situation; however, in that case both methods showed minor differences between the two invasive decapod species. Nonetheless, since food availability can change in time and functional responses can show considerable intraspecific variability (Iacarella et al. 2015a; 2015b; Iltis et al. 2018), it seems likely that the relationship between the results of the two methods can be different even among the same species depending on the circumstances.

Conclusion

In summary, combining functional responses and stable isotope analysis allowed us to draw robust conclusions on the trophic ecology of the species and also provided further insights into the trophic structure of the studied ecosystem. We recommend the use of this approach in further studies which might also shed more light on the factors determining how predatory potentials are realized in natural environments.

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Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

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Author contributions

Conceptualization: PB, KP, CFV. Formal analysis: KP, AB, PB. Funding acquisition: PB. Investigation: KP, PB, AB. Methodology: AB, PB, KP, CFV. Visualization: PB, CFV, KP. Writing - original draft: PB, KP. Writing - review and editing: AB, CFV.

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Data availability

All of the data that support the findings of this study are available at: https://figshare.com/projects/Functional_responses_and_stable_isotope_analysis_of_invasive_Ponto-Caspian_mysids/177159.

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Supplementary material 1

Estimates of the differences between the parameters of the Type II functional response curves with and without mature specimens (*a*: attack rate, *h*: handling time)

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Data type: docx

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